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Requirements of Carp and Rainbow Trout for Essential Amino Acids*1

Chinkichi OGINO*2

The requirements of carp and rainbow trout for essential amino acids (EAA) were determined based on the daily increase of each EAA in the body of these fish species when they were fed on the diets containing proteins with high biological values. As the amounts of EAA increased daily in 100g of the body weight correspond approximately to the requirements for EAA of these fish species per 100g of the body weight per day, these amounts of EAA should be supplied from the dietary proteins. It was found that there were no distinct differences in the EAA requirements between carp and rainbow trout. The contents of EAA in the dietary proteins (%) needed to satisfy the requirements of carp and rainbow trout were; leucine, 4.1, 4.4, isoleucine, 2.3, 2.4, valine, 2.9, 3.1, threonine, 3.3, 3.4, phenylalanine, 2.9, 3.1, tyrosine, 2.0, 2.1, methionine, 1.6, 1.8, cystine, 0.8, 0.9, tryptophan, 0.6, 0.5, arginine, 3.8, 3.5, histidine, 1.4, 1.6, and lysine, 5.3 and 5.3, respectively, when diets containing 40% protein of an 80% digestibility were supplied at a feeding rate of 3% of the body weight.

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The essential amino acid requirements of fish are found by rearing fish on feed in which the totality or a part of the feed proteins has been replaced by amino acid mixtures through the relationship between the amounts of the various essential amino acids in the feed and the weight increase rates of the reared fish[1-3]). Since feed proteins are absorbed after hydrolization into amino acids in the digestive tract, it would seem that when fish ingest proteins, and when they ingest amino acid mixtures, there should be a great difference in the absorption speed of amino acids by the digestive tract. It is therefore not clear whether the essential amino acid requirements found through feed employing amino acid mixtures may be applied to the case of ingestion of proteins.

In the present report, we used the essential amino acid compositions of fish body proteins as standard to find the essential amino acid requirements with regard to carp and rainbow trout. That is, it seems that when fish are reared on feed containing proteins whose biological value is high, the amount of essential amino acid increase in the body of the fish indicates the essential amino acid requirement of the fish. We sought the protein essential amino acid compositions satisfying these requirements in conjunction with the volumes of proteins contained in the feed, the feeding rates and the digestive absorption rates of proteins.

Methods of the tests

Feed composition

We used feed containing at least 33% protein of high biological

value for carp and rainbow trout[4]). The protein source was denatured with hot alcohol[4]). The feed composition was as stated in our former report.

Rearing method

We performed rearing by the running water method (200 mL/min) while aerating inside a glass water tank (30 x 45 x 30 cm). The water temperature was 20-25°C for the carps and 15-18° C for the rainbow trouts.

Measurement of the N increase

We measured the total N of the fish body for each type of feed at the start of the rearing as well as 14 and 28 days later, and found in this way the increase in N. Thinking that N in the body of the fish increased in proportion to its weight, we sought the increase per day per 100 g.

Method of analysis

We analyzed the amino acids with an amino acid analytic meter (from Nippon Denki Co.; model JLC-6AS model). We measured the tryptophan by the colorimetric method[6]. We measured N by the Kjeldahl method. To know the amino acid composition of the proteins in the body of the fish, we took 10 fish weighing 3-5 g, boiled them 3 times for 2 hours each with ethyl alcohol, removed the soluble matters, dried and pulverized after

defatting with ether, and obtained the final results by using the hydrochloric acid hydrolyzates.

Table 1. Determination of daily increase of nitrogen in the body of carp

Dietary protein source	Dietary protein level (%)	Days of feeding	Total weight		Nitrogen increased (g)	Daily increase of nitrogen (mg/100 g wt)
			Initial (g)	Final (g)		
Egg albumin	33.0	14	74.5	122.7	1.23	88.5
〃	33.0	14	76.4	125.7	1.26	88.5
〃	33.0	14	62.1	95.1	0.86	77.8
〃	33.0	14	62.7	96.8	0.88	78.5
〃	33.0	14	61.1	97.1	0.93	83.5
〃	33.0	28	74.5	197.2	3.02	79.2
〃	33.0	28	76.4	201.8	3.09	79.2
〃	33.0	28	62.1	154.6	2.29	75.3
〃	33.0	28	62.7	156.1	2.30	75.0
〃	33.0	28	62.1	157.6	2.38	77.5
Average						80.3±4.7

Table 2. Determination of daily increase of nitrogen in the body of rainbow trout

Dietary protein source	Dietary protein level (%)	Days of feeding	Total weight		Nitrogen increased (g)	Daily increase of nitrogen (mg/100 g wt)
			Initial (g)	Final (g)		
Egg yolk	34.1	20	69.9	148.1	1.78	81.5
Whole egg	35.6	20	68.4	145.4	1.75	81.5
Egg albumin	37.5	20	68.4	153.8	1.94	87.0
Muscle*1	33.2	20	68.1	142.9	1.69	79.5
Muscle*2	36.7	20	69.8	153.1	1.89	85.0
Casein	37.2	20	68.4	139.8	1.62	78.0
Casein	36.5	19	125.5	251.6	3.10	86.3
Casein-Muscle*3	35.9	19	125.3	232.5	2.64	77.8
Casein-Whole egg*4	35.8	19	127.1	249.3	2.81	78.4
Casein	36.5	10	125.8	174.1	1.27	80.5
Average						81.5±3.2

*1 Muscle protein of rainbow trout.
*2 Muscle protein of common squid, *Todarodes pacificus*.
*3 A 1: 1 mixture of casein and the squid muscle protein.
*4 A 1: 1 mixture of casein and whole egg protein.

Results and discussion

The increases of N in the fish body

Table 1 shows the results obtained with regard to increases in N for

1.5 g - 1.9 g carps reared 14 and 28 days on feed in which the protein source was egg albumin. The N increase per 100 g of body weight was on average 80.3 $\pm$ 4.7 mg per day. According to the results obtained by rearing 2 g -- 4 g rainbow on feeds containing different kinds of protein (Table 2), the average per day was 81.5 $\pm$ 3.2 mg. Since the real N increase in the fish body is the value obtained by adding the elimination volumes of the metabolic as well as endogenous N (14 mg for carp and 9.5 mg[7]) for rainbow trout per day and per 100 g of body weight) to the above measured values, the total is 94.3 mg for carps and 91.0 mg for rainbow trouts. In other words, one may say that when carrying out rearing with feed containing at least 33% of good quality protein, the increase in N per day and 100 g of body weight is almost the same with regard to carps and rainbow trouts of 1.5 - 4 g. This is why we thought that 93 mg per day should be about right for both species of fish and why we adopted this value. If we convert to protein, the increase in protein corresponds to 0.58 g per day per 100 g of body weight.

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Essential amino acid requirements

When fish are reared on feed containing proteins which have a high biological value, the proteins increase by 0.58 g per day per 100 g of body weight in growing carps and rainbow trouts. As the essential amino acid composition of the increased proteins is believed to be extremely close to the essential amino acid composition of the total fish proteins, one may say that the various essential amino acid volumes in 0.58 g of protein found from this (relation) indicate the various essential amino acid requirements per day and per 100 g of body weight with regard to

carps and rainbow trouts. These are shown in Table 3.

Table 3. The contents of essential amino acids (EAA) in the whole body-proteins of carp and rainbow trout and their daily increase

Amino acids	Carp		Rainbow trout	
	Contents in whole body protein (%)	Daily increase of EAA*1 (g/100 g wt)	Contents in whole body protein (%)	Daily increase of EAA*1 (g/100 g wt)
Leucine	6.9	0.040	7.2	0.042
Isoleucine	3.8	0.022	4.0	0.023
Valine	4.8	0.028	5.2	0.030
Threonine	5.3	0.031	5.5	0.032
Phenylalanine	4.8	0.028	5.1	0.030
Tyrosine*2	3.2	0.019	3.4	0.020
Methionine	2.6	0.015	2.9	0.017
Cystine*2	1.2	0.007	1.3	0.008
Tryptophan	1.0	0.006	0.9	0.005
Arginine	6.3	0.037	5.9	0.034
Histidine	2.3	0.013	2.5	0.015
Lysine	8.7	0.050	8.7	0.050

*1 These values correspond to the requirements of these fish species for EAA (g amino acid/100 g body weight/day).
*2 These amino acids are not essential for these fish species.

Table 4. The contents of EAA in the dietary proteins needed to satisfy the requirements for EAA of carp and rainbow trout when dietary protein levels are 35 and 40%, the digestibility, 80 and 90%, and the feeding rate, 3% of the body weight, respectively. Values indicate % amino acids in crude protein

	Carp				Rainbow trout			
	35% protein diet		40% protein diet		35% protein diet		40% protein diet	
Digestibility (%)	90	80	90	80	90	80	90	80
Leucine	4.2	4.8	3.7	4.1	4.4	5.0	3.9	4.4
Isoleucine	2.3	2.6	2.0	2.3	2.4	2.8	2.1	2.4
Valine	3.0	3.4	2.6	2.9	3.2	3.6	2.8	3.1
Threonine	3.3	3.8	2.9	3.3	3.3	4.1	3.0	3.4
Phenylalanine	3.0	3.4	2.6	2.9	3.2	3.6	2.8	3.1
Tyrosine	2.0	2.3	1.8	2.0	2.1	2.4	1.9	2.1
Methionine	1.6	1.8	1.4	1.6	1.8	2.3	1.6	1.8
Cystine	0.8	0.9	0.7	0.8	0.9	1.0	0.8	0.9
Tryptophan	0.7	0.8	0.6	0.6	0.6	0.6	0.4	0.5
Arginine	3.9	4.4	3.3	3.8	3.6	4.0	3.1	3.5
Histidine	1.3	1.5	1.2	1.4	1.6	1.8	1.4	1.6
Lysine	5.3	6.0	4.7	5.3	5.3	6.0	4.7	5.3

Minimum essential amino acid contents in feed proteins

The essential amino acid composition in feed proteins needed to

satisfy the essential amino acid requirements is believed to vary depending on the volume of feed given, the contents of proteins in the feed and on the protein digestion-absorption rate. If the feeding rate is set at 3% of body weight per day (the average during growth seems to be about 3%) the essential amino acid composition of the feed proteins required to satisfy the requirements of carps and rainbow trouts is as shown in Table 4 when the feed protein contents are 35 and 40%, and if the protein digestion-absorption rates are 80 and 90%. Almost no difference between carps and rainbow trouts was noted with regard to the essential amino acid composition (minimum contents) of the proteins needed to satisfy the requirements. We noted no great differences between the values for chinook salmon [1-3], carp*1 and eel*2 measured by using amino acid mixtures. We thus saw that the differences are small even when the measurements were based on the use of feed containing amino acid mixtures or when measuring by the present method.

In the former report we compared the nutritive value of caseine to that of chicken egg proteins, of rainbow trout as well as of sagittated calamary muscle, and found it to be somewhat inferior. The main reason seemed mainly to lie in a lower total volume of sulfur-containing amino acids and in lower arginine contents[4]. On the basis of the present

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*1 K. Nose: Collected Summaries of the Convention of the Japanese Society of Scientific Fisheries 149 (1976, April); 241 (1976, October); 603 (1977, April)

*2 S. Nii: same source, 151 (1976, April); 302 (1978, April)

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results, the arginine contents (4.1%) of caseine are close to the values indicated in Table 4, and it seems that depending on the rearing conditions there may be shortages. Among the sulfur-containing amino acides, methionine had contents (2.3%) close to the values in Table 4, but the cysteine contents (0.3%) are clearly insufficient. Consequently, the main factor determining the nutritive value of caseine seems to be the total volume of sulfur-containing amino acids.

Table 5. The contents of EAA in the diets of carp and rainbow trout needed to satisfy their requirements when the dietary protein level is 40% and the protein-digestibility, 80%. The values on chinook salmon, carp, and eel by other workers are listed for comparison.

Amino acid	Carp (%)	Rainbow trout (%)	Chinook salmon[16] (%)	Carp*1 (%)	Eel*1 (%)
Leucine	1.64	1.76	1.6	1.25	2.0
Isoleucine	0.92	0.96	0.9	0.95	1.5
Valine	1.16	1.24	1.3	1.4	1.5
Threonine	1.32	1.36	0.9		1.5
Phenylalanine	1.16	1.24	2.1(Tyr, 0%)	1.0(Tyr, +)	1.2(Tyr, 2%)
Tyrosine	0.80	0.84			
Methionine	0.64	0.72	1.6(Cys, 0%)	0.8(Cys, 2%)	0.9(Cys, 1%)
Cystine	0.32	0.36			
Tryptophan	0.24	0.20	0.2	0.3	0.53
Arginine	1.52	1.40	2.4	1.65	2.3
Histidine	0.56	0.64	0.7	0.8	1.34
Lysine	2.12	2.12	2.0	2.2	2.5

*1 T. Nose: Abstracts of the Annual Meeting of the Japan. Soc. Sci. Fish., 149 (1976, Apr.); 241 (1976, Oct.); 603 (1977, Apr.).
*2 S. Arai: Abstracts of the Annual Meeting of the Japan. Soc. Sci. Fish., 131 (1976, Apr.); 302 (1978, Apr.).

The lowest essential amino acid contents in feed

Table 5 shows the essential amino acid contents of feed obtained from the values in Table 4 when the protein contents in the feed are set at 40% and if the digestion-absorption rate of the proteins is set at 80%. Table 5 shows also the values for chinook salmon, eel and carp obtained with feed containing amino acid mixtures. We see that there is no big difference in the minimum contents of essential amino acid in feed

with regard to the various species of fish. The amino acid composition of the total fish body proteins or muscle proteins hardly changes[8]) during the growth of the fish. However, as no great difference is observed between the fish species[9]), there should be no great difference in the minimum contents of essential amino acids in the feed required to satisfy the requirements. It is well known that the phenylalanine requirements vary depending on the tyrosine contents, and that the methionine requirements vary depending on the caseine contents. The presently obtained results show the minimum contents of phenylalanine and methianine when tyrosine and cysteine are contained.

I wish finally to thank Dr. Kenzo SOTOYAMA, assistant professor at the Tokyo Fisheries College who helped me in the analysis of the amino acids.

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